ANNEX D TO DOD SMALLPOX RESPONSE PLAN 14 June 2002
SPECIMEN-COLLECTION GUIDELINES

REFERENCES.

a. CDC Interim Smallpox Response Plan, Guide D, Specimen Collection Guidelines, 23 Jan 02. http://www.bt.cdc.gov/DocumentsApp/Smallpox/RPG/GuideD/Guide-D.doc.

b. CDC Laboratory Response Network (LRN). Protocol for Interim Guidelines for Collection and Shipment of Specimens from Suspected Smallpox Patients, 15 Apr 02.

1. General. This DoD Annex augments CDC Guide D. Appendix D-1 summarizes CDC Guide D and this DoD Annex on one page.

a. Mission. Military medical treatment facility (MTF) clinical laboratories participate in the Laboratory Response Network (LRN), a collaborative effort of the Centers for Disease Control & Prevention (CDC) and the Association of Public Health Laboratories (www.bt.cdc.gov/LabIssues/index.asp). These laboratories maintain personnel trained to process clinical-microbiology specimens and detect suspicious microbes that might indicate use of bioterrorism agents. Accordingly, they will follow CDC guidance for collecting and processing specimens. MTFs will be prepared to collect and ship clinical specimens for further laboratory evaluation, as specified in this annex.

b. Assumptions. Not applicable.

c. Planning Factors.

(1) MTF clinical laboratories are designated as Level A and Level B laboratories within the LRN (see also Appendix D-2.

(2) Recommended procedures at various biosafety levels are described in Appendix D-3.

d. Coordinating Instructions. With a suspected case of smallpox, inform command channels as soon as possible. MTF headquarters will promptly submit a Serious Incident Report to their higher headquarters and report to other public-health authorities, as specified in Annex A. After consultation with the CDC, the MTF will collect appropriate samples for testing by CDC, the US Army Medical Research Institute of Infectious Diseases (USAMRIID), or another member of the LRN designated by the CDC. If the case is considered high risk for smallpox, no additional clinical specimens will be collected from that person for testing within the hospital or clinic laboratory. CDC or the local MTF will notify the Federal Bureau of Investigation (FBI), so the FBI can arrange for transport of the specimen(s) to the LRN laboratory designated by the CDC.

e. Legal Considerations. Early in a smallpox outbreak, maintain a chain-of-custody record for critical specimens, to allow for their use as forensic evidence of the use of a bioweapon.

2. Execution.

a. Concept of Operations. Training providers in identification of smallpox is a key requirement to ensure MTFs minimize any unreasonable suspicion. In a case where smallpox is possible, notify local experts as soon as possible to determine if the case warrants a smallpox work-up. Once CDC is notified, their Rapid Response and Advanced Technology Laboratory may send a team to assist. If not, and the case is warranted, then a specimen-collection team identified by the MTF commander and trained on smallpox collection techniques should collect these specimens. At no time should a health-care provider request the laboratory undertake isolation or identification of variola virus (smallpox). This virus is a biosafety level 4 (BSL-4) agent (Appendix D-3). Avoid any possibility of viral spread to the general population. In the future, CDC will recognize specific BSL-3 laboratories with the capability to identify smallpox virus. It is not appropriate to perform laboratory tests outside strict containment conditions to rule out any other virus in a suspected smallpox case. Instead, the CDC will receive the specimens and perform the testing. The CDC is the primary site for all smallpox testing. The US Army Medical Research Institute of Infectious Diseases (USAMRIID) is a backup facility in case of surge demand for smallpox testing. CDC will coordinate with USAMRIID if it needs assistance.

b. Tasks and Responsibilities.

(1) MTF Commander:

(a) The MTF commander is responsible for training health-care providers on the symptoms for smallpox and notification procedures for a suspected smallpox case.

(b) The MTF commander is responsible for providing a smallpox specimen-collection team with necessary supplies (Appendix D-4). As smallpox vaccinations resume, give priority to these collection teams. Until then, use proper personal protective equipment.

(c) The MTF Commander is responsible for ensuring the clinical laboratory is trained and qualified to provide proper shipping of smallpox specimens, as well as removal of cadavers with suspected or known smallpox infection.

(2) The initial health-care provider: Any provider who suspects a diagnosis of smallpox in a patient will notify the MTF commander immediately. The MTF will implement infection-control measures consistent with Annex C.

(3) Specimen-Collection Team: Each MTF will appoint and train a smallpox specimen-collection team. Appendix D-5 below specifies in detail the procedures for the

collection and shipment of specimens from suspected smallpox patients. These teams will document training in procedures identified in Appendix D-5. MTFs will document periodic competency assessment (e.g., quarterly) and confirmation of adequate smallpox specimen-collection supplies and protective equipment.

(a) Only recently (within 3 years), successfully vaccinated personnel wearing appropriate barrier protection (e.g., gloves, gown, shoe covers, protective eyewear) will be involved in specimen collection for suspected cases of smallpox. Respiratory protection is not needed for personnel with recent, successful vaccination. Masks and eyewear or face shields should be used if splashing is anticipated.

(b) If unvaccinated personnel must collect specimens, use only those without contraindications to vaccination, as they would require immediate vaccination if the diagnosis of smallpox is confirmed. Fit-tested N95 masks should be worn by unvaccinated individuals caring for suspected patients.

(c) These teams will document training in specimen-collection procedures. MTFs will document periodic competency assessment (e.g., quarterly) and confirmation of adequate smallpox specimen-collection supplies and protective equipment.

(4) The Laboratory Medical Director, Department of Pathology, will ensure that laboratory personnel responsible for shipping specimens are trained on shipping procedures and maintain inventories of proper shipping supplies (Appendix D-6, Appendix D-7). The laboratory must have a person trained and certified in shipment of hazardous substances including infectious disease shipping. This person must be recertified every two years. The US Army Center for Health Promotion and Preventive Medicine (USACHPPM) provides such training ("Transport of Biomedical Materials"), as may other Services or Agencies. Information on this training is provided on the CHPPM web page at http:/chppm-www.apgea.army.mil/TrainCon/datePage.aspx. The Medical Director is also responsible for following guidelines in Appendix D-5 for collecting and disposing of autopsy specimens, as well as transporting an autopsy cadaver.

c. Reporting. Will be performed as indicated in Annex A, as well as via Serious Incident Reports through command channels.

3. Administration and Logistics.

a. Supply and Storage. The MTF will maintain a smallpox specimen-collection kit at all times, inspected at least every six months to ensure no items have outdated. Materials required for specimen collection from each patient are listed in Appendix D-4.

b. Equipment. The MTF will provide fitted N-95 masks or higher or HEPA-filtered respirators (with appropriate fit-testing before operational use) to personnel designated as part of the smallpox specimen-collection teams. See also Annex C.

c. Transportation. MTFs will arrange transportation of smallpox specimens (biological agents) according to International Air Transport Association (IATA) guidelines. A copy of the Dangerous Goods Regulations (DRG) can be obtained by calling 1-800-716-6326 or through the Internet at www.iata.org or www.who.int. Biological agents are considered hazardous materials and their transportation is subject to regulatory control. Appendix D-5 directs the detailed procedures for packaging possible smallpox samples. A designated person in the MTF laboratory must be certified in infectious-disease shipping. Prior coordination is required with personnel at the local Transportation Office or the Air Transportation Operations Center (ATOC) to ensure shipping requirements are followed for movement by military air or ground transportation.

4. Command, Control, & Communications. Communication about a suspected smallpox case cannot be delayed. Notification of higher headquarters is required. If at anytime an MTF Level A laboratory has difficulty in communicating to a civilian laboratory, they should notify their regional Level B laboratory for assistance.

5. Special Situations.

a. Specimen collection in U.S. European Command (EUCOM). Landstuhl Regional Medical Center (LRMC) has a BSL-3 laboratory. A microbiologist there is trained on CDC LRN procedures and guidelines. This person can assist with specimen collection, transportation, and notification of the Department of State and the FBI, for further shipment to an LRN laboratory designated by the CDC. Additionally the Technical Escort Unit (TEU) may be available for sample transport. Coordination with the TEU flows from the MTF commander to the Unified Command Surgeon's Office.

b. Specimen collection in U.S. Pacific Command (PACOM). Tripler Army Medical Center currently has a BSL-2 laboratory, with BSL-3 capabilities planned for FY03. A microbiologist there is trained on CDC LRN procedures and guidelines. This person can assist with specimen collection, transportation, and notification of the Department of State and the FBI, for further shipment to an LRN laboratory designated by the CDC. Additional, the Navy Environmental & Preventive Medicine Unit—6 (NEPMU-6) at Pearl Harbor has a BSL-2 laboratory and can also support processing suspicious samples/substances.

c. Troops deployed outside CONUS in a theater in conflict. Field Medical Facilities should inform their command of any suspected smallpox case. Isolate this patient from unvaccinated people. If the theater is near to a fixed MTF with an LRN laboratory, that MTF will provide assistance. If this is not feasible, the Command Surgeon for that theater should develop an appropriate detailed concept of operations for biological threat agents in the medical annex. A designated Army Special Medical Augmentation Response Teams (SMART), Air Force Infectious Disease Team (ID Team), or other Service equivalent teams may be able to provide collection and shipping capabilities. Deployable assets such as the Theater Army Medical Laboratory (TAML), the Navy Forward Deployed Laboratory, or the Air Force Biological Augmentation Team (BAT), and special theater or regional fixed-site laboratories such as those supporting bio-

identification for biosensor systems in U.S. Central Command (CENTCOM) and PACOM are options for theater operations.

APPENDIX D-1
Specimen Collection -- Summary

1. Laboratories at military treatment facilities (MTFs) belong to either level A or level B of the Laboratory Response Network (LRN), coordinated by the Centers for Disease Control & Prevention (CDC).

2. Each military treatment facility (MTF) will periodically train its laboratory staff in the collection, handling, and shipping of possible smallpox-infected specimens.

4. MTFs will maintain a supply of shipping materials suitable for shipping smallpox-infected specimens.

5. All specimens will be shipped to either a member of the Laboratory Response Network (LRN) designated by CDC. The Federal Bureau of Investigation (FBI) oversees shipments of possibly smallpox-infected specimens.

6. Early in a smallpox outbreak, collect specimens with preservation of forensic evidence in mind. Maintain a chain-of-custody record for critical specimens.

7. Overseas laboratories (e.g., Landstuhl, Tripler, NEPMU-6) can provide support.

APPENDIX D-2
Levels in the Laboratory Response Network (LRN).

1. Level A Laboratories – Standard clinical laboratories. Bio-Safety Level 2 (BSL-2). Typical roles: detect early (presumptive cases) of disease, minimal identification of agents. Rule-out or Refer. Role vis-à-vis smallpox—None.

2. Level B Laboratories – Reference laboratories (e.g., state health departments, larger military medical centers). Bio-Safety Level 2 or 3 (BSL-2 or -3). Typical roles: perform identification, confirmation, and susceptibility testing. Isolate. Rule-in and Refer. Role vis-à-vis smallpox—None.

3. Level C Laboratories – Reference laboratories (e.g., CDC, Armed Forces Institute of Pathology, large facilities laboratories with advanced capacity for testing, some molecular technologies). Bio-Safety Level 3 (BSL-3). Typical roles: rapid identification. Rule-in and Refer. Role vis-à-vis smallpox—In future, at BSL-3.

4. Level D Laboratories – Bio-Safety Level-4 Laboratories (i.e., CDC, USAMRIID). Typical roles: high-level characterization, special surge capacity and advanced molecular typing techniques. Probe for universe of agents. Role vis-à-vis smallpox—definitive diagnosis.

Source: Centers for Disease Control & Prevention,
www.bt.cdc.gov/LabIssues/index.asp.

APPENDIX D-3
Recommended Biosafety Levels for Infectious Agents

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II Biological Safety Cabinets (BSCs) or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; personal protective equipment (PPE): laboratory coats; gloves; face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of lab clothing before laundering • Baseline serum	Primary barriers = Class I or II BSCs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems • Other requirements outlined in the text

Source: Department of Health & Human Services. Biosafety in Microbiological and Biomedical Laboratories, 4th ed, May 1999. http://bmbl.od.nih.gov/

APPENDIX D-4
Supplies Needed for Specimen Collection.

Some or all of the following materials will be required for specimen collection from each patient.

Disposable protective latex or vinyl gloves (sterile gloves not required)
Disposable protective gowns
N-95 masks or higher properly fitted HEPA-filtered respirators (see below)
Protective eyewear
Shoe covers
Biohazard plastic disposable bags
Disposable scalpel with No. 10 blade
Several sterile 26-gauge needles
3.5 or 4 mm punch biopsy kit
Needle driver
Suture
Suture removal kit
4 to 8 sterile dry polyester or cotton swabs
4 clean plastic or glass microscope slides
4 plastic single-slide holders
2 or more electron microscopy grids
Electron microscopy quality forceps
Electron microscopy grid box
Eight 1.5 to 2.0 ml sterile screw-capped plastic vials (Sarstedt with o-ring)
5 or 10 ml syringe with 18- or 20-gauge needle
1 Vacutainer holder
2 Vacutainer needles (20 x 1 ½ in.)
One 10 ml marble-topped Vacutainer tubes, **or** one 10 ml yellow-topped serum-separator tube for serum collection (plastic tube preferable)
One 5 ml purple-topped tubes for whole blood buffy-coat collection for viral isolation (plastic tube preferable)
Parafilm

APPENDIX D-5
Specimen Collection Procedures.

1. General Considerations.

a. Because of the risk of breakage, avoid glass containers whenever possible. Use plastic vials, bottles, or slide holders as the primary container for all specimens.

b. Each patient's lesion specimens must be packaged separately from other patient specimens to avoid cross-contamination.

c. All procedures involving handling potentially infectious material should be performed in laboratories utilizing Biosafety Level 2 or 3 practices. Any activity that brings hands or fingers in contact with mucosal surfaces, such as eating, drinking, smoking, or applying make-up should be avoided. Thorough hand-washing using soap or soap containing Lysol or soaps such as *Hibiclens* should be done before leaving the laboratory. Areas of the skin known to have come in contact with virulent variola or monkeypox virus should be washed with soap and decontaminated with 0.5% sodium hypochlorite with at least a 1 minute contact time. Administration of smallpox vaccination, and possibly VIG, should be determined in coordination with CDC.

d. In the event of a large outbreak of confirmed smallpox, other laboratories with smallpox diagnostic capabilities may used to meet diagnostic surge demand. CDC will designate these laboratories. Instructions for sending specimens to these laboratories will be given at the time of their designation.

2. Specimen-Collection Procedure for Patients with Vesicles or Pustules. Blood samples from people with severe, dense smallpox rash may be difficult to draw, as the skin may slough off. A central line may be needed for access, in cases where a peripheral blood draw is difficult.

a. Put on protective equipment described above

b. Use scalpel (or a sterile 26-gauge needle) to open, and remove, the top of the vesicle or pustule and place the skin of the vesicle top into a 1.5-2 mL screw-capped plastic tube. Allow the material to dry. Label the tube as outlined below**.**

c. Scrape the base of the vesicle or pustule with the blunt edge of the scalpel, or with the wooden end of an applicator stick or swab and do the following:

(a) Apply a microscope slide to the vesicular fluid multiple times, with progressive movement of the slide, to make a touch prep.

(b) Allow the fluid to air-dry for 10 minutes, without smearing.

(c) Label the slide as outlined below.

(d) Store the dried slide in a plastic slide container

(e) Store slides in plastic slide holders for shipping. Parafilm may be used to wrap the slide holder to prevent accidental opening. Store slides from different patients in separate plastic slide holders to prevent cross contamination.

(f) If a slide is not available, swab the base of the lesion with a polyester or cotton swab, place in a screw-capped plastic vial, break off applicator handle, and screw on lid. Do not add transport medium to the vial. Label the container as outlined below.

d. If available, lightly touch an electron microscope grid to the unroofed base of the lesion and allow to air dry. Repeat this procedure two more times, varying the pressure applied to the unroofed lesion (lighter or firmer pressure). Place in grid box and record which slot is used for each patient specimen.

e. Biopsy two vesicles with 3.5 or 4 mm punch biopsy kit.

(1) Place one biopsy specimen in formalin.

(2) Place one biopsy specimen in a 1.5-2 mL screw-capped container. Do not add any fluid.

(3) Label the containers as outlined below.

f. Draw 10 ml of blood into a plastic marble-topped tube, or a plastic yellow-topped serum-separator tube. Label the tube as outlined below and place in collection bag. If plastic tubes are not available:

(1) Draw blood into a glass marble-topped or yellow-topped serum-separator

(2) Label glass tube as outlined below and place glass tube into a Styrofoam protector for packaging and shipping.

g. Test in Validation: Swab or brush posterior tonsillar tissue, then break off end of applicator into a 1.5-2 mL screw-capped tube. Do not add transport medium. Label the tube as outlined below.

h. Test in Validation: Draw 5 ml of blood into plastic purple-topped tube. Gently shake the tube containing the blood to mix the tube contents and prevent clotting of blood. Label the tube as outlined below and place in collection bag. If plastic tubes are not available:

(1) Draw blood into a glass purple-topped tube.

(2) Gently shake tube to mix the contents

(3) Label tube as outlined below and place in Styrofoam protector for packaging and shipping.

i. Label all samples as follows:

(1) Patient name.

(2) Date of collection.

(3) Source of specimen (vesicle, pustule, or scab).

(4) Social Security number or date of birth of patient (for cross-referencing specimens).

(5) Name or initials of person collecting specimen.

(6) If patient is hospitalized, include hospital identification number (e.g., surgical pathology number).

j. Place specimens from a single patient into a biohazard bag with an outside label that includes:

(1) Patient name.

(2) Date of collection.

(3) Social Security number or date of birth of patient.

k. Package specimens from a single patient (except biopsies):

(1) On gel packs at 4°C.

(2) In appropriate bio-safety shipping containers in a manner to withstand shocks, pressure changes, or other conditions incident to ordinary handling in transportation.

(3) In a manner to avoid leakage of contents.

l. Package non-formalin lesion biopsy for shipping on dry ice, leave formalin-fixed biopsy at room temperature. DO NOT FREEZE formalin-fixed biopsy sample.

m. Specimens may be stored in conditions outlined above, if shipped within 24 hours of collection. If this is not possible, store samples on dry ice or at –20°C to -70°C until, and through, shipment. A key exception applies to electron microscope grids and

serum, which should remain at 4°C. If there will be a delay in shipping, spin serum to separate from clot, store at 4°C, and ship at 4°C.

n. Final instructions regarding transportation will be given at the time of consultation and may involve a personal escort carrier to ensure sample tracking and integrity.

o. After specimen collection is completed, all protective materials worn by the specimen collector (e.g., gloves, mask, gown) and all used sample collection materials (e.g., Vacutainer holders, swabs) must be placed in red biohazard bags and autoclaved or incinerated prior to disposal. Dispose of needles in an appropriate sharps container.

3. Specimen-Collection Procedure for Patients with Scab Lesions.

a. Put on protective equipment as outlined above and use a 26-gauge needle to pick or pry off as many scabs as possible (at least four).

b. Place two scabs in each of two screw-capped plastic 1.5 to 2 mL vials.

c. Biopsy two lesions with 3.5 or 4 mm punch biopsy kit.

(1) Place one biopsy specimen in formalin

(2) Place one biopsy specimen in a 1.5 to 2 mL screw-capped container.

(3) Label the containers as outlined below.

d. Draw 10 ml of blood into a plastic marble-topped tube, or a plastic yellow-topped serum-separator tube. Label the tube as outlined below and place in collection bag. If plastic tubes are not available:

(1) Draw blood into a glass marble-topped or yellow-topped serum-separator tube.

(2) Label glass tube as outlined below and place glass tube into a Styrofoam protector for packaging and shipping.

e. Test in Validation: Swab or brush posterior tonsillar tissue, then break off end of applicator into a 1.5 2 mL screw-capped tube. Do not add transport medium. Label the tube as outlined below.

f. Test in Validation: Draw 5 ml of blood into plastic purple-topped tube. Gently shake the tube containing the blood to mix the tube contents and prevent clotting of blood. Label the tube as outlined below and place in collection bag. If plastic tubes are not available:

(1) Draw blood into a glass purple-topped tube.

(2) Gently shake tube to mix the contents.

(3) Label tube as outlined below and place in Styrofoam protector for packaging and shipping.

g. Label all samples as follows:

(1) Patient name**.**

(2) Date of collection.

(3) Source of specimen (vesicle, pustule, or scabs).

(4) Social Security number or date of birth of patient (for cross-referencing specimens).

(5) Name or initials of person collecting specimen.

(6) If patient is hospitalized, include hospital identification numbers (e.g., surgical pathology number).

h. Place specimens from a single patient into a biohazard bag with an outside label that includes:

(1) Patient name.

(2) Date of collection

(3) Social Security number or date of birth of patient.

i. Package specimens from a single patient (except for biopsies):

(1) On gel packs at 4°C.

(2) In appropriate bio-safety shipping containers in a manner to withstand shocks, pressure changes, or other conditions incident to ordinary handling in transportation.

(3) In a manner to avoid leakage of contents.

j. Package non-formalin lesion biopsy specimens for shipping on dry ice. Package formalin-fixed biopsy specimens at room temperature. DO NOT FREEZE formalin-fixed biopsy samples.

k. Specimens may be stored in conditions outlined above, if shipped within 24 hours of collection. If this is not possible, store samples on dry ice or at –20°C to –70°C

(except for serum and formalin-fixed biopsy specimens). If there will be a delay in shipping, spin serum to separate from clot, store at 4°C, and ship at 4°C.

l. Final instructions regarding transportation will be given at the time of consultation and may involve a personal escort carrier to ensure sample tracking and integrity.

m. After specimen collection is completed, all protective materials worn by the specimen collector (e.g., gloves, mask, gown) and all used sample collection materials (e.g., Vacutainer holders, swabs) must be double-bagged in red biohazard bags and autoclaved or incinerated prior to disposal. Needles should be disposed of in an appropriate sharps container.

4. Autopsy Specimens.

a. Freeze autopsy specimens for virus isolation, including portions of skin containing lesions, liver, spleen, lung, lymph nodes, and/or kidney. Ship these specimens on dry ice.

b. Formalin-fixed tissue is suitable for histopathology, immunohistochemistry and PCR, but should not be frozen and must be packaged separately from autopsy specimens for virus isolation (which must be frozen). All major organs (e.g., liver, spleen, skin, lung, lymph nodes, kidney) should be adequately sampled and submitted for evaluation.

c. Specimens should be labeled and packaged for transport as outlined above.

d. After specimen collection, all non-reusable specimen collection and barrier protection materials should be placed in red biohazard bags and autoclaved prior to disposal. All re-usable autopsy equipment must be autoclaved or disinfected according to standard laboratory procedures before re-use.

e. Extreme precautions are necessary to prevent dissemination of smallpox virus during an autopsy. Standard precautions should be observed for all contact with the body. Contact the CDC (NCID Division of Healthcare Quality Control at 404-639-6413 or Pathology Activity 404-639-3133) before an autopsy, to review the containment features of individual autopsy suites, procedures for autopsy, and disinfection after an autopsy. To transport the body to the autopsy suite, wrap the body in a large, impervious plastic bag, or a disaster pouch, sealed airtight with tape. The body should be sealed in a second large, impervious plastic bag before transportation to the autopsy suite. Ideally, the autopsy would be performed in a room with negative air pressure with respect to the surrounding facilities. All doors and windows of the autopsy rooms should be closed during the autopsy, and the air exhausted must not be recirculated. Only necessary personnel with up-to-date vaccination (within 3 years) should participate in the autopsy. Vaccinated personnel should wear disposable clothing, gowns, gloves, caps, booties, and masks and face shields or protective eyewear to prevent splashing of the mucus membranes. No personal clothing should be worn. All clothing articles from

the autopsy room should be placed in biohazard bags and autoclaved or incinerated. After autopsy, the body should be double-bagged in another set of large, impervious plastic bags. If vaccination before autopsy is not possible, unvaccinated personnel should perform the autopsy wearing, in addition to the protective garments above, respiratory protection (e.g., HEPA-filtered breathing apparatus or a self-contained breathing apparatus).

APPENDIX D-6
Specimen Shipping.

1. Guidelines for Packaging and Transporting Biological Agents.

a. Biological agents include infectious agents of humans, plants, and animals, as well as the toxins that may be produced by microbes and by genetic material potentially hazardous by itself or when introduced into a suitable vector. Etiologic agents and infectious substances are closely related terms that are found in the transfer and transportation regulations. Biological agents may exist as purified and concentrated cultures, but may also be present in a variety of materials such as body fluids, tissues, and soil samples. Biological agents and the materials known or suspected to contain them are recognized by federal and state governments as hazardous materials and their transportation and transfer is subject to regulatory control.

b. Transportation refers to the packaging and shipping of these materials by air, land, or sea, generally by a commercial conveyance. Transfer refers to the process of exchanging these materials between facilities.

2. Transportation. Regulations on the transportation of biological agents are aimed at ensuring that the public and the workers in the transportation chain are protected from exposure to any agent that might be in the package. Protection is achieved through:

a. the requirements for rigorous packaging that will withstand rough handling and contain all liquid material within the package without leakage to the outside,

b. appropriate labeling of the package with the biohazard symbol and other labels to alert the workers in the transportation chain to the hazardous contents of the package,

c. documentation of the hazardous contents of the package should such information be necessary in an emergency situation, and

d. training of workers in the transportation chain to familiarize them with the hazardous contents so as to be able to respond to emergency situations.

3. Regulations.

a. U.S. Public Health Service. 42 CFR Part 72. Interstate Transportation of Etiologic Agents. This regulation is in revision to harmonize it with the other U.S. and international regulations. http://www.cdc.gov/od/ohs/biosfty/shipregs.htm

b. U.S. Department of Transportation. 49 CFR Parts 171-178. Hazardous Materials Regulations. Applies to the shipment of both biological agents and clinical specimens. http://hazmat.dot.gov/rules.htm.

c. U.S. Postal Service. 39 CFR Part 111. Mailability of Etiologic Agents. Codified in the Domestic Mail Manual 124.38: Etiologic Agent Preparations. http://www.access.gpo.gov or www.usps.gov.

d. Occupational Health and Safety Administration (OSHA). 29 CFR Part 1910.1030. Occupational Exposure to Bloodborne Pathogens. Provides minimal packaging and labeling requirements for transport of blood and body fluids within the laboratory and outside of it. http://www.osha.gov/comp-links.html.

e. Dangerous Goods Regulations (DGR). International Air Transport Association (IATA). These regulations provide packaging and labeling requirements for infectious substances and materials, as well as clinical specimens that have a low probability of containing an infectious substance. These are the regulations followed by the airlines. These regulations are derived from the Committee of Experts on the Transport of Dangerous Goods, United Nations Secretariat, and the Technical Instructions for the Transport of Dangerous Goods by air that is provided by the International Civil Aviation Organization (ICAO). http://www1.iata.org/NR/ContentConnector/CS2000/SiteInterface/pdf/cargo/dg/43rev8April22.pdf.

f. Importation of Etiologic Agents of Human Disease. 42 CFR Part 71 Foreign Quarantine. Part 71.54 Etiologic Agents, Hosts and Vectors. This regulation requires an import permit from the Centers for Disease Control and Prevention for importing etiologic agents of human disease and any materials, including live animals or insects, that may contain them. An application and information on importation permits may be obtained by calling 1-888-CDC-FAXX and enter document number 101000 or on the Internet at: http://www.cdc.gov/od/ohs/biosfty/imprtper.html. See also http://www.unm.edu/~sheaweb/sheamanual/biosfty/biosaf_i.htm.

g. Importation of Etiologic Agents of Livestock, Poultry and Other Animal Diseases. 9 CFR Parts 92, 94, 95 96, 122 and 130. These regulations requires an import permit from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services to import or domestically transfer etiologic agents of livestock, poultry, other animals, and any materials that might contain these etiologic agents. Information may be obtained at (301) 734-3277, or from the Internet at: http://aphisweb.aphis.usda.gov/ncie.

h. Transfer of Select Biological Agents of Human Disease. Regulations on the transfer of biological agents are aimed at ensuring that the change in possession of biological materials is within the best interests of the public and the nation. These regulations require documentation of the personnel, facilities, and justification of need for the biological agent in the transfer process and subsequent approval of the transfer process by a federal authority. The following regulations fit in this category: 42 CFR Part 72.6 Additional Requirements for Facilities Transferring or Receiving Select Agents. Facilities transferring or receiving select agents must be registered with the CDC and

each transfer of a select agent must be documented. Information may be obtained on the Internet at: http://www.cdc.gov/od/ohs/lrsat.

i. Export of Etiologic Agents of Humans, Animals, Plants and Related Materials. Department of Commerce. 15 CFR Parts 730 to 799. This regulation requires that exporters of a wide variety of etiologic agents of human, plant and animal diseases, including genetic material, and products which might be used for culture of large amounts of agents, will require an export license. Information may be obtained by calling the DoC Bureau of Export Administration at 202-482-4811 or through the Internet at: http://bxa.fedworld.gov, or http://www.bxa.doc.gov.

APPENDIX D-7
Specimen Packaging.

1. General Packaging Requirements for Transport of Biological Agents and Clinical Specimens. Figures 1 and 2 illustrate the packaging and labeling of infectious substances and clinical specimens in volumes of less than 50 ml, in accordance with the provisions of subparagraph 72.3(a) of the regulation on Interstate Shipment of Etiologic Agents (42 CFR, Part 72). A revision is pending that may result in additional package labeling requirements, but this has not been issued in final form as of the publication of this fourth edition of *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

a. Figure 1 below shows the generalized "triple" packaging (i.e., primary receptacle, water tight secondary packaging, durable outer packaging) required for a biological agent of human disease or materials known or suspected of containing them. This packaging requires the "Infectious Substance" label shown in Figure 2 on the outside of the package. This packaging must be certified to meet rigorous performance tests as outlined in the DOT, USPS, PHS, and IATA regulations.

b. Clinical specimens with a low probability of containing an infectious agent are also required to be "triple" packaged, but performance tests require only that the package shall not leak after a 4-foot drop test. DOT, PHS, and IATA require a "clinical specimen" label on the outside of the package.

c. The shipper's name, address and telephone number must be on the outer and inner containers.

2. Shipping suspected biological threat agents to the CDC.

a. Use the following address: Centers for Disease Control and Prevention, 1600 Clifton Road NE, ATTN: DASH (forward to RRAT Lab), Atlanta, GA 30333.

b. For shipping questions relating to sending specimens to the CDC, call (404) 639-2888.

Figure 1. Packing and Labeling of Infectious Substances

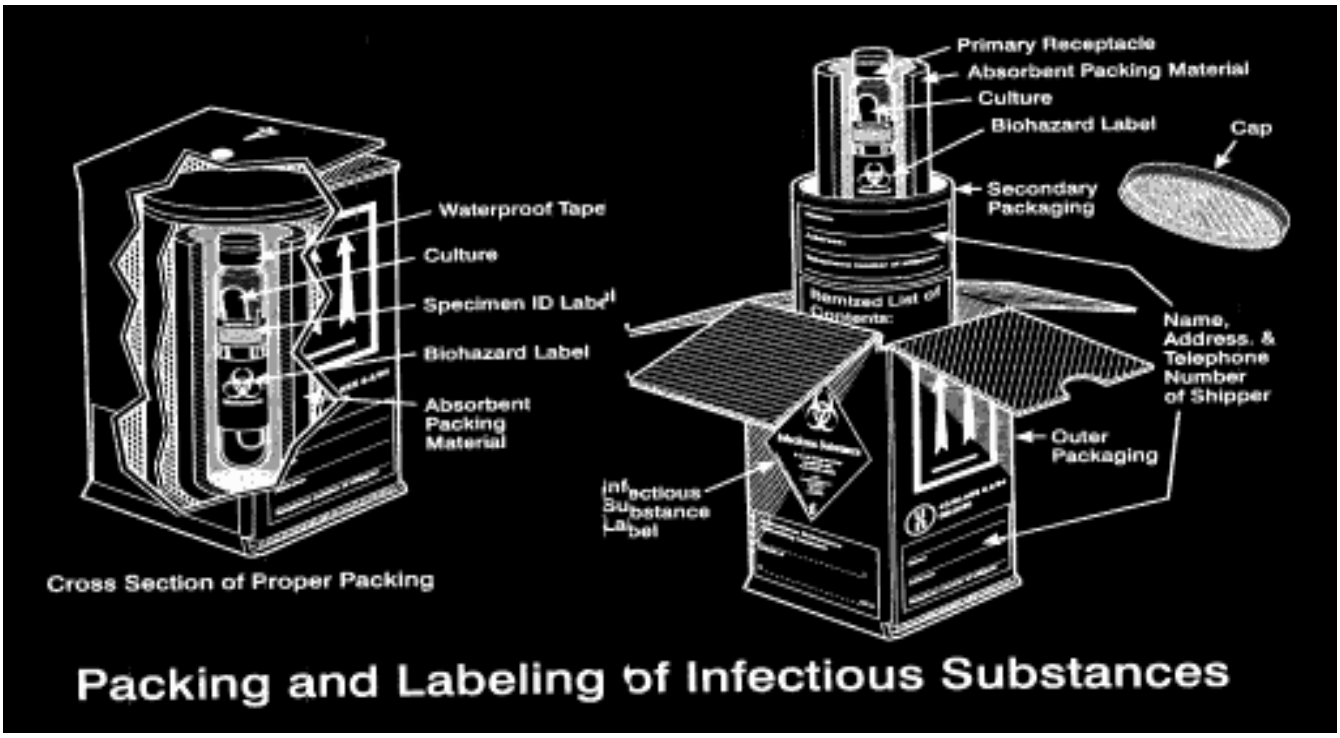

Figure 2. Packing and Labeling of Clinical Specimens